STUDIES ON THE PLANT CELL.—VIII.[1]

BRADLEY MOORE DAVIS.

SECTION VI. COMPARATIVE MORPHOLOGY AND PHYSIOLOGY OF THE PLANT CELL.

WE shall devote this section to the discussion of a number of topics some of which have received brief mention in the preceding papers of the series but with other subjects will now be considered in some detail. The material will be treated under the following five headings:—

1. The simplest types of plant cells.
2. Comparisons of the structures of some higher types of plant cell with simpler conditions.
3. Some apparent tendencies in the evolution of mitotic phenomena.
4. The essential structures of the plant cell and their behavior in ontogeny.
5. The balance of nuclear and cytoplasmic activities in the plant cell.

1. THE SIMPLEST TYPES OF PLANT CELLS.

There are three groups of plants which are conspicuous for the simplicity of their cell structure. They are: the Cyanophyceæ (blue-green algæ), Schizomycetes (bacteria), and the Saccharomycetes (yeasts). All three groups have received much attention and there has accumulated an extensive literature which we shall not attempt to treat in detail, since it has been handled very fully by the specialists in these subjects. We shall, however, present the most important conclusions and

[1] This paper concludes the series of studies on the plant cell. The author has a number of complete sets of reprints of this and the earlier sections. Enquiries may be addressed to Professor Bradley M. Davis, University of Chicago.

try to give the present status of investigations in these most difficult subjects.

Cyanophyceæ (*Blue-green Algæ*).— The most recent and comprehensive papers on the cell structure of the Cyanophyceæ are by Fischer ('97), Macallum ('99), Hegler (: 01), Bütschli (: 02), Kohl (: 03), Zacharias (: 00, : 03), and Olive (: 04). Olive gives an especially clear analysis of the situation in this field of investigation at the present time and an excellent historical review of earlier literature may be found in Hegler (: 01). The discussions center chiefly around (1) the presence or absence of a nuclear structure and its behavior in cell division, (2) the distribution of the blue-green pigment (phycocyan) and the structure of a possible chromatophore, and (3) the nature of certain conspicuous inclusions within the cell, called cyanophycin granules and slime globules. An outline in tabular form of the views of some thirty investigators on these subjects is given by Olive (: 04, p. 10).

Writers from the earliest periods of cell studies on the Cyanophyceæ have recognized the presence of a central body in the interior of the cell more or less sharply differentiated from the peripheral region, which holds the coloring matter and certain inclusions. The central body contains granular material which stains and behaves in other particulars like chromatin. But as a rule this granular material is not confined within a membrane or vacuolar cavity which has proved the most serious difficulty to its acceptance as chromatin and the central body as a nucleus. Then many investigators have not been able to satisfy themselves that the central body exhibits the phenomena characteristic of nuclear division even in a simple form. Consequently much doubt has been expressed as to its morphology and possible relation to a nucleus.

The most recent and detailed investigations have, however, brought forward much evidence to the effect that the granular material in the central body is chromatin which becomes organized into chromosomes that are distributed by a form of mitotic division. In the vegetative cells, which generally divide rapidly, the chromatin is never held within a nuclear membrane but in young heterocysts and spores such inclosing membranes have been found (Olive, : 04).

Olive (:04) has given especial attention to methods of sectioning and staining on the slide and presents the most detailed account of the structure and behavior of the chromatin and the simple apparatus which brings about the division of the central body. The central body is made up chiefly of dense kinoplasm with a fibrillar structure in which lie chromosomes that may be counted and whose number is found to be constant in several species. Thus there are eight chromosomes in a species of Glœocapsa and Nostoc and sixteen in certain forms of Oscillatoria, Phormidium, and Calothrix. The chromatin in some cases was observed to be organized into what seemed to be a simple type of spirem (especially clear in Glœocapsa) within the central body, and the chromosomes are formed by a concentration of material at certain points which are constant in the cells of the same plant.

Olive found evidence that the chromosomes split during the process of division of the central body and are gathered in two groups at the ends of the achromatic structure which is generally flattened at the poles and conforms in other particulars to the shape of the cells. The two sets of chromosomes are finally separated by the cell wall which develops from the periphery during cell division and cuts the achromatic structure in the middle region. That portion of the central body which remains between the two sets of daughter chromosomes is regarded by Olive as equivalent to the central spindle so well defined in stages of anaphase and telophase in mitoses of higher plants. The central body during this process of division has certainly very much the appearance of a simple type of spindle although there are not present the large fibers so characteristic of nuclear figures in higher plants. Moreover it can scarcely be held that the division is one of simple fusion when chromosomes are present in constant numbers and split into two groups with each division of the cell. Olive believes that the achromatic structure, present during cell division, is a disc-shaped, generally flat-poled spindle, densely fibrous in structure and that the fission of the chromosomes and their separation into two sets constitutes a true mitotic division of the central body, which is a nucleus.

Other authors as Scott ('88), Hegler (:01), Bütschli (:02), and Kohl (:03), also believe that the central body is a nucleus which divides mitotically but none has described the process as so closely similar to nuclear division in higher plants as in the account of Olive. Some of their results are criticized by Olive as based on preparations in which the stain was not properly differentiated or the sections were too thick. Among the recent writers Wager (:03) stands alone as holding that the nucleus divides directly (amitotically) by a process of simple fission. Both Kohl and Wager conceive the chromatin as in a network or convolute spirem which breaks up into segments which are drawn apart, thread by thread, quite a different process from the splitting of organized chromosomes. Other authors have held that the granules in the central body were chromatin although they were not willing to admit the structure as a nucleus. Thus Macallum ('99) found that the central body contained phosphorous and "masked iron" to a conspicuous degree and he, with other investigators, has shown that this structure resists the action of artificial gastric juice, solutions of pepsin, etc. These chemical reactions are considered confirmatory of the theory that the granular material is a proteid of a high order of organization such as would be expected of chromatin. However, such chemical tests are very difficult to apply and do not seem to the writer so important in establishing the nature of the central body as does the careful study of its structure and activity during cell division. The objection that the central body lacks a membrane, universally present around resting nuclei of higher plants, is not regarded as vital by Olive. In the first place such a membrane may be found around the resting nuclei in young heterocysts and spores and its absence in vegetative cells is probably explained by the rapidity of the successive cell divisions. There are some recent writers, as Massart (:02) and Zacharias (:00, :03) who are still unconvinced that the granules in the central body are chromatin and that the structure is the equivalent of a nucleus. Their papers and figures, however, clearly show that they have failed to find the detailed structures of other investigators.

Fischer ('97) has been the most conspicuous opponent of the

view that the cells of the Cyanophyceæ and also of the Schizomycetes contain nuclei, taking a position in sharp opposition to that of Bütschli ('96). Fischer's conclusions were based on his failure to find that differentiation of the protoplasm within the cell demanded by the conception of the central body and the activities of this structure during cell division as described by other authors. He presented a sharp criticism of the conclusions based on the reaction of stains in determining the nature of protoplasmic structures, criticisms largely directed against the investigations of Bütschli. He showed by some ingenious experiments upon emulsions of albumen fixed on a slide that stain reactions were a purely physical phenomenon. Thus the same combinations of stains, such as saffranin and gentian violet, may be made to give exactly opposite results in differentiating a mixture of large and small globules of albumen when used in reverse order. He attached no importance to the so called affinity of a protoplasmic structure for a particular stain and would not accept such apparent affinity as evidence of its chemical nature. The fact that the central body takes chromatic stains did not seem to him important evidence of its nuclear character and he was very positive in his belief that the cells of the Cyanophyceæ do not contain nuclei and that the central bodies should not be considered the phylogenetic forerunners of such structures.

This attitude of Fischer towards conclusions based on stain reactions was later presented in more elaborate form in his critique ('99) on methods of fixing and staining protoplasm and has had an important influence on methods of cytological investigation and interpretation. The stain reaction is now regarded as probably merely a physical phenomenon but an effective means of differentiating protoplasmic structures. The determination of their morphology rests with an understanding of their history and behavior in the activities of the cell. Although Fischer's general criticism of methods of cell research was timely and in some instances richly deserved, nevertheless his particular conclusions respecting the cell structure of the Cyanophyceæ and the Schizomycetes have not been sustained by investigators who have followed the history of the protoplasmic structures in the cells of these organisms.

We may pass now to the peripheral region of the cell which holds the blue-green coloring matter of the Cyanophyceæ. A number of investigators, as Wager (:03), Kohl (:03), Hegler (:01), and Hieronymus ('92), have held that this pigment was contained in minute granules distributed throughout the cytoplasm under the cell wall. These granules have at times been termed chromatophores or plastids and Hegler has proposed for them the name cyanoplastids. Other authors, especially Fischer ('97), Nadson ('95), Palla ('93), and Zukal ('92) have been unable to find these color-bearing granules and have believed the coloring matter to be uniformly diffused throughout the peripheral region of the cell. Fischer has made a particularly thorough study of the reactions of the pigmented region to various acids in comparison with the chromatophores of higher algæ and concludes that no plastids are present but that the color is held in a hollow cylindrical or spherical outer layer of protoplasm which may be termed a chromatophore. Olive supports Fischer, approaching the subject from a very different point of view. If minute plastids are present they should be visible in fixed and stained material and Olive is unable to find any trace of Hegler's cyanoplasts. The granules of the outer region of the protoplast seem to be colorless inclusions.

Perhaps the most confused part of the discussion on the structure of the cell of the blue-green algæ is that which deals with certain inclusions. There are apparently two sorts which are very common in the cells: (1) the cyanophycin granules (Borzi) and (2) the slime globules. The cyanophycin granules are very apt to lie along the cross walls in filamentous forms or in other peripheral regions of the cell. They are generally believed to be a form of food material and it has been suggested that they are the first visible product of photosynthetic processes, but their chemical nature is under dispute. The slime globules lie more frequently in the interior region of the cell close to the nucleus and frequently within this structure. They have been termed nucleoli by some authors and also confused with chromatin. Besides these two bodies, other minute globules have been described as oil or fat and some remarkable crystalloid structures have been figured, especially by Hieronymus ('92).

Indeed the entire subject is so confused that it does not seem desirable for us to take it up in detail at this time, especially since these inclusions are apparently all secretions or excretions and not morphological features of the cell. The most comprehensive discussions of the subject will be found in the papers of Hegler (: 01), Kohl (: 03), and Zacharias (: 03).[1]

Schizomycetes (*Bacteria*).—The history of research upon the cell structure of the Schizomycetes has run in large part parallel with that on the Cyanophyceæ. The clearest results have come from studies upon the larger forms of the sulphur bacteria, especially certain species of Beggiatoa, and on certain forms of Spirillum. The more minute types and pathogenic forms in particular have proved very baffling because of their small size and it can scarcely be said that we fully understand their cell structure. As in the Cyanophyceæ, investigators of the bacteria fall into two groups: one holding that the Schizomycetes entirely lack a nucleus and the other that there is present a structure, often termed a central body, which is the equivalent of a nucleus.

Bütschli ('96, : 02) has been the most conspicuous advocate of the latter view. He described and figured clearly a central body in the cells of Beggiatoa, Chromatium, and Spirillum with the same organization as given in his account of that body in the Cyanophyceæ. The central body contains granular material which Bütschli regards as chromatin and the structure is shown in stages of division. Bütschli has no hesitation in giving the central body the value of a nucleus. It lies within a peripheral

[1] Since the above was written a lengthy paper by Fischer, "Die Zelle der Cyanophyceen" has appeared (*Bot. Zeit.*, vol. 63, p. 51, 1905), too late to be included in these reviews. Fischer has not changed his conclusions on the chief points as discussed in his earlier papers. The chromatophore is a closed cylindrical structure; the cyanophycin granules are proteid in character; glycogen and another carbohydrate, anabænin, are conspicuous substances in the cell; the central body is not a nucleus but the seat of important metabolic processes concerned with these carbohydrates, and its contents and behavior in cell division have only a superficial resemblance to nuclear structure and mitosis; the chromatin granules of Bütschli, Olive, and others are masses of anabænin (a carbohydrate). Fischer's criticisms are fundamental and it is evident that the morphologists must clearly establish the proteid nature of the central body and its contents (especially the "so called" chromatin granules) before they can expect the acceptance of their conclusions as to its nuclear character.

region of protoplasm as in the Cyanophyceæ. There is of course no blue-green pigment (phycocyan) in the cells of bacteria and consequently no chromatophore but several sorts of inclusions may be present in the protoplasm. The nature of some of the inclusions is not clear and this subject has not been given as much attention as in the Cyanophyceæ. It is significant that this cell structure should be found so clearly in the Beggiatoa since this organism seems very close to Oscillatoria in its morphology. Some of the larger species of Beggiatoa may be expected to yield conclusions similar to those of Olive's investigation on Oscillatoria if sectioned and critically stained, especially as the cells are very large in some forms and there is probably less extraneous matter to complicate the interpretation of the preparations.

As has been stated, investigations upon the smaller species of bacteria and especially upon pathogenic forms have met with great difficulties. These led at one time to the ingenious theory of Bütschli ('90), followed by Zettnow ('97) that possibly the entire protoplast had the value of a nucleus. That is to say, an outer peripheral region of cytoplasm had either never been developed in these organisms or, if present, had become so closely associated with the chromatin that it could not be distinguished as a special region of the cell. A peripheral region of cytoplasm is represented in some of the larger forms by the cilia and by accumulations of protoplasm at the ends of the cells, especially clearly shown in Spirillum (Bütschli, '96; Zettnow, '97). Later Zettnow ('99) and Feinberg (:00) applying the staining method of Romanowski, followed by several later investigators with improved technique (Nakanishi, :01, and others), succeeded in differentiating a minute body in the cells of smaller bacteria and pathogenic forms, which is regarded now as similar to the central body of the sulphur bacteria and a true nucleus. This structure is very minute since it occupies a portion of these exceedingly small cells. Naturally it will be very difficult to obtain any detailed knowledge of its structure and behavior during cell division. But enough seems to be known to justify the belief that differentiated nuclear structures are probably present even in the smallest types of bacteria. A recent paper

of Vejdovsky (: 04) describes and figures a simple type of spindle in *Bacterium gammeri* and *Bryodrilus ehlersi* with a separation of two groups of chromatin granules during mitosis.

The chief critics of the conclusions that the cells of Schizomycetes are nucleated have been Migula ('95) and Fischer. The latter author in particular has devoted considerable attention to the group especially in his paper of 1897 which is largely a discussion of Bütschli's ('96) results on studies of the blue-green algæ and bacteria. Fischer considers the central body described by Bütschli in the sulphur bacteria as merely a vacuolate region of the cell made conspicuous by the arrangement of the sulphur grains and that the structure does not appear in cells which are free from sulphur. The granular material, considered as chromatin by others, is regarded by Fischer as reserve material. The central body described by Bütschli in the cells of Spirillum is stated to be a product of contraction. In general the same criticism which Fischer applied to the methods of staining and interpretation of structures in the Cyanophyceæ is presented for the Schizomycetes. Fischer cannot justify Bütschli's ('90) view that the smaller bacteria are chiefly composed of nuclear substance, a view which probably has few if any followers to-day and could scarcely claim to be more than a passing suggestion. In short, Fischer finds no evidence of a nuclear structure in the Schizomycetes but curiously ends by declaring that the group has no affinities with the Cyanophyceæ but that its forms are closely associated with the Flagellata.

Saccharomycetes (*Yeasts*).— The structure of the yeast cell has been perhaps the subject of as long a series of investigations as the cells of the Cyanophyceæ and Schizomycetes, and the problems in both cases have many similar features. The chief problem in the yeasts has concerned the presence or absence of an organized nucleus or its equivalent in the form of some simpler structure. The accounts range from a complete denial of its presence to descriptions of a nuclear apparatus of considerable complexity which passes through some rather involved activities during cell division. It is impossible for us to treat the subject historically. We shall only consider the accounts of the most recent investigators and try to determine the probable

bearing of these studies. An admirable review of the early literature is presented in Wager's paper of 1898.

Wager ('98) himself has made one of the most detailed studies of the yeast cell and his conclusions on the presence of a "nuclear apparatus" will be made the starting point of our discussion. The yeast cell contains a structure, termed by Wager a "nuclear body," generally situated at one side, close to the cell wall. This body resembles the nucleolus of higher plants in its homogeneous structure and reaction to stains. Besides the "nuclear body" Wager finds a vacuole always present which contains granular material and is an important part of the nuclear apparatus. This "nuclear vacuole" must be carefully distinguished from other vacuoles of the usual type which merely contain glycogen. There are besides some globular bodies in the protoplasm whose nature may be oil in some cases and proteid in others. The "nuclear body" is always in close contact with the "nuclear vacuole" but is never within it. The amount of granular material in the nuclear vacuole is variable but it sometimes contains a dense mass. This content is believed to be chromatin from the behavior to stains and insolubility in digestive fluids. Sometimes the nuclear vacuole disappears but in such cases the granular network is found in contact with the nuclear body and sometimes distributed about it in a very regular manner. The chromatic granular material appears then to be a permanent substance in the cell and always closely associated with the nuclear body, sometimes distributed about it and sometimes included within a special vacuole.

Wager concludes that the nuclear apparatus consists of (1) a nucleolus (nuclear body) and (2) a store of chromatin in a network, either enclosed in a vacuole in close contact with the nucleolus or lying freely about the nucleolus or sometimes disseminated in granules generally throughout the cytoplasm. Wager believes that the nuclear vacuole arises from the fusion of numerous small vacuoles which lie around the chromatin granules which thus come to lie within a common vesicle. This mode of origin seems reasonable from what we know of the history of the nuclear vacuole which arises around the chromosomes that gather at anaphase of mitosis to form daughter

nuclei in higher plants. The earlier investigators for the most part failed to recognize the chromatic granules and network and considered the nucleolar body (nucleolus) to be the nucleus of the cell. Janssens and Leblanc ('98), however, described a nucleus with a membrane containing caryoplasm and a nucleolus, and other authors noted the vacuole and believed that it held some relation to the nucleus.

Both the nuclear vacuole and the nuclear body (nucleolus) take part in the process of bud formation. The bud appears on the opposite side of the cell from the nuclear body and the nuclear vacuole lies between. The bud contains at first cytoplasm alone; then the nuclear vacuole begins to pass into it and the nuclear body takes a position in the vicinity, between the mother-cell and the bud. The nuclear body now divides by simple fission and one half enters the bud. The nuclear vacuole gradually constricts and is drawn apart in the canal between the two cells. The two daughter nuclear vacuoles and nuclear bodies then pass to opposite ends of the mother- and daughter-cells respectively. If the nuclear vacuole is absent the chromatin network is drawn apart so that a division is effected in a similar manner.

At the time of spore formation, the chromatin is reported by Wager to become so closely associated with the nuclear body that the two substances cannot be easily separated and behave as one. The resultant structure elongates and divides by constriction and the subsequent divisions are of the same character. Strands of deeply staining protoplasm between the daughter nuclei are of interest as suggesting the possibility of a simple type of spindle. Wager describes the formation of spore walls around the nuclei enclosing a portion of the protoplasm and thus cutting the spores out from the remaining non-nucleate cell contents. The details of this process are not known and might prove very interesting since the process, from Wager's account, would seem to be one of free cell formation without, however, the characteristics described by Harper in spore formation within the ascus. It should be more thoroughly studied for it is possible that the division will be found to involve cleavage furrows and really prove to be a type of segmentation by constriction (Section II, *Amer. Nat.*, vol. 38, p. 453, June, 1904).

Several papers have appeared on the structure of the yeast cell since Wager's account of 1898. Marpmann (:02) and Feinberg (:02) described much simpler conditions than are reported by Wager, and recognize scarcely more than a deeply staining body which they term a nucleus. Hirschbruch (:02) gives an extraordinary description, accompanied by diagrammatic figures, of a nuclear structure and a body, staining red and blue respectively, which are supposed to fuse previous to the development of a bud, but the account is so unsatisfactory as to merit little attention. Janssens (:03) reviews the work of these investigators and others in comparison with his earlier results (Janssens and Leblanc, '98). Guilliermond (:04) has published the most recent paper presenting more completely his conclusions of an earlier investigation in 1902.

Guilliermond's conclusions have some points of resemblance to those of Wager. He finds a nuclear vacuole containing a granular network believed to be chromatin and a nucleolar structure. The entire body seems to be a true nucleus, not differing in its essentials from the nuclei of other fungi. Sometimes all the material in the nucleus seems to be condensed into a central body, a sort of chromatin nucleolus (chromoblast) somewhat resembling a similar structure in Spirogyra. Guilliermond figures the nucleus as constricting during the process of budding, one part passing into the daughter cell. His figures show clearly deeply stained material outside of the nuclear membrane in a position similar to that of Wager's nucleolar body (nucleolus).

These points of agreement seem to justify at least in part Wager's account, but of course the peculiarities of both lead one to suspect that there are important features in the structure of the nucleus and in the events of nuclear division which have not been determined. It certainly seems probable that chromatin is present in definitely organized bodies (chromosomes) sometimes within a vacuole and sometimes lying around a nucleolar structure. The latter also holds an intimate relation to the chromatin, which is frequently true in higher plants. There are indications that a simple type of spindle is present at least in the nuclear divisions during spore formation. In view of

Olive's results in studies on the Cyanophyceæ it does not seem unreasonable to hope that more accurate staining of very thin sections will bring the peculiarities of these accounts into harmony with mitotic phenomena of higher forms.

The accounts of conjugation in yeasts (Barker, :01 and Guilliermond, :03) which were discussed under "Asexual Cell Unions and Nuclear Fusions" in Section IV give no additional information on the essential structure of the yeast cell.

2. Comparisons of the Structure of some Higher Types of Plant Cell with Simpler Conditions.

Some of the most fruitful and interesting fields of investigation in cell structure are likely to be in those border groups between the very simplest conditions of the lower algæ and fungi and the higher regions where the nucleus and processes of mitosis have clearly the essential features which are generally ascribed to this structure and its activities. At present the gap seems very great between the simple conditions of the Schizophyta and the groups of algæ and fungi on the next higher general level. But as a matter of fact we know almost nothing of the nuclear structure in the lowest groups of the Chlorophyceæ, *i. e.*, among the simplest of the unicellular green algæ. It is rather remarkable that this region should have been so neglected.

The Nucleus.— Comparative studies on the nucleus naturally treat chiefly of the chromosomes and nucleolus. One of the most interesting features of more recent research on the nucleus has been the steady accumulation of evidence indicating that the nucleolus holds a very important relation to the chromatin content. There are types among the lower algæ in which the whole or a greater part of the chromatin is gathered into a dense nucleolar body in the resting nucleus. Spirogyra is the best known illustration of this condition and has been studied by several investigators. Similar phenomena have been reported by myself in Corallina (Davis, '98), by Golenkin ('99) for Sphæroplea, and by Wolfe (:04) for Nemalion. Some nuclei, however, particularly in the higher plants have nucleoli whose

substance does not seem to contribute directly to the chromosomes and these have been regarded as secretions within the nucleus. Strasburger believed that such were masses of reserve material drawn upon by the kinoplasm during the process of spindle formation. The term plastin has been applied to such substance in the nucleolus and also in the linin as cannot be directly connected with chromatin. A nucleolus may consist of plastin alone, or have with this substance varying quantities of chromatin. Nucleoli consisting of chromatin alone may be expected among the lower plants from the studies on Spirogyra, Corallina, Sphæroplea, and Nemalion. Plastin and chromatin are probably closely related substances.

A recent paper of Wager (:04) indicates that the nucleolus of some higher plants holds a far closer relation to the chromosomes than has been supposed and rather weakens Strasburger's theory of the structure as a reserve mass drawn upon during mitotic activities. This study and recent papers by Miss Merriman (:04) and Mano (:04) have all been upon the cells of root tips while the conceptions of Strasburger and others have been founded largely on the structure and behavior of the nucleolus in the spore mother-cell during the mitoses of sporogenesis. Wager treats of the root tip of Phaseolus, Miss Merriman of Allium, and Mano of Solanum and Phaseolus. They are important contributions to the subject of the nucleolus and should be considered in any treatment of this structure. The papers appeared too recently to be noted in our brief account of the nucleolus in Section I which is consequently incomplete. Wager's paper especially presents an excellent review of the literature on the nucleolus in the plant cell.

Wager concludes that the nucleolus is really a portion of the nuclear network and that the spirem is derived in part at least from this structure. Material from the nucleolus then passes into the chromosomes. Also, in the reconstruction of the daughter nuclei the chromosomes are massed together at a certain stage and from this mass the nucleolus emerges, taking out with it the greater part of the chromatin. Wager then considers the nucleolus as a store of chromatin which must be taken into account in theories of heredity based on the morpho-

logical independence of the chromosomes. Miss Merriman reports the origin of the nucleoli as masses among the meshes of chromatin from which they draw their substance. Mano, in contrast to Wager, holds that the nucleoli appear as globules independent of the chromatin network and later flow together into a single body. The chromosomes are also believed by Mano to be morphologically independent of the nucleolus and if the latter furnishes material to the former it is not by the emergence of strands as described by Wager. Mano then holds the nucleolus to be an accessory structure without morphological relation to the chromosomes.

The theory of the individuality of the chromosomes is of course vitally concerned with the problem of the morphology of the nucleolus but this topic we have reserved for later treatment under the caption : "The Essential Structures of the Plant Cell and their Behavior in Ontogeny." The chromatin and nucleoli within the nucleus of a higher plant lie in a vacuole whose fluid content is bounded by a plasma membrane similar to that around any vacuole in the cell. Lawson (: 03) and Grégoire and Wygaerts (: 03) have emphasized this structural condition in recent papers but the central idea seems to be an old one running through the writings of Strasburger from an early period.

We bring up these striking conceptions of nuclear structure in the higher plants because it seems very probable that a much clearer understanding of the problems will come through investigations upon the simpler conditions in the lower plants. There, we may hope to find evidence of the primitive forms of nucleolar and chromatic associations with perhaps some clues as to the manner of the development of the higher types of structure. Thus the yeast cell, as reported by Wager ('98) with its chromatin sometimes collected within a vacuole and sometimes distributed in the cytoplasm and a nuclear body (nucleolus) in close association with the nuclear vacuole, but not within, is of the greatest interest as presenting intermediate stages in the complexity of nuclear structure and illustrates what may be hoped from further research among the lower forms.

The Chromatophore and Plastid.— In considering the great variety of chromatophores and plastids exhibited among the

thallophytes one notices at once certain features of their distribution in various groups. The large chromatophores are characteristic of the cells of simpler and more primitive groups and the small plastids, numerous in the cells, are generally present in types which are at a fairly high evolutionary level. There are exceptions of course to this general statement but some of these are probably significant of phylogenetic relations.

The evidence all indicates that the primitive type of chromatophore was a large structure in the peripheral region of the protoplast and with an ill defined boundary or occupying the entire surface of the cell. This type of structure is at present characteristic of chromatophores of the Cyanophyceæ and is also present in numbers of the lower groups of green algæ. Thus we may find many types in the Pleurococcaceæ whose cells contain a pigment so diffused that it is impossible to establish definite limits and similar conditions often appear in the cells of some of the higher algæ as in Hydrodictyon and certain simple forms of the Ulothricaceæ.

The simple diffused types of chromatophores of the lower algæ become replaced in higher groups either by sharply differentiated structures of definite form and often showing internal organization in the form of pyrenoids or by numerous plastids. There is considerable evidence that the plastids have arisen by the successive splitting or division of large organized chromatophores. The most highly differentiated chromatophores are found in the Conjugales and the remarkable size and symmetry of these cells is emphasized by the same peculiarities of the chromatophores. They are generally so placed in the cells as to give an almost perfect balance of protoplasmic structure. This principle is especially clearly illustrated among the desmids and in such forms as Zygnema and Mougeotia while even Spirogyra illustrates the principle strikingly in the distribution of its spirally wound chromatophores.

Plastids are characteristic of the Siphonales, Charales, most of the Rhodophyceæ, the higher Phæophyceæ, and all groups generally above the thallophytes. It seems to be the type of structure best suited to cell activities since with few exceptions it is found in groups in the highest lines of plant evolution in

various directions. The only striking exceptions to this broad principle are Anthoceros, whose cells contain each a single large chromatophore, and Selaginella. Selaginella is especially interesting for, while the cells of the meristematic region and young organs contain but a single chromatophore, this structure may divide later in some types to form a chain of discoid plastids in older cells connected with one another by delicate strands of protoplasm. Thus in the life history of certain species of Selaginella we have plainly shown the change from a single chromatophore to a number of plastids. It seems probable that this history repeats in general outline the evolutionary history of the condition characterized by numerous plastids within a cell from a primitive type of cell structure with but a single chromatophore. Anthoceros and Selaginella may be regarded as forms whose cells still retain the primitive conditions with respect to the single large chromatophore. There are somewhat similar illustrations in the Rhodophyceæ as in Nemalion and Batrachospermum whose cells hold a single large chromatophore while most of the more highly organized red algæ have numerous plastids. A beautiful series of stages illustrating the evolutionary principles outlined above might be worked out in the Phæophyceæ.

What is the fundamental principle underlying the substitution of numerous plastids in a cell in place of a single chromatophore? The author believes that it must have relation to the preservation within large cells of a certain balance of the metabolic centers. The fission of a plastid is a process of constriction and studies on Anthoceros (Davis, '99, p. 94) indicate that the bounding cytoplasmic membrane exerts pressure upon the elongating structure. It seems probable that the division is due to the mechanical separation of material that is too bulky for the most effective results of photosynthesis which in the case of a single chromatophore are centered in a particular region of the cell. By the division of a chromatophore into numerous plastids the photosynthetic activities are distributed among several centers and a much better balance results within the cell. It is very interesting that the large elaborate chromatophores with their peculiar internal differentiations, the pyre-

noids and caryoids, should have been displaced by the much simpler and apparently homogeneous plastids.

A comparative study of chromatophores and plastids from the point of view of their evolutionary history is much to be desired and such research would necessitate extensive studies among the lower groups of algæ and especially in the Protococcales. Such studies would involve far more than the general morphology of the chromatophore and plastid. The structure and activities of the pyrenoid are a very important subject as shown by the investigations of Timberlake on Hydrodictyon and nothing is known of the function of the caryoid. A detailed investigation of the chromatophore or plastid throughout ontogeny is yet to be made.

The Cytoplasm.— There is no region of the plant cell whose structure is more varied and as little understood as that presented by the cytoplasm with its diverse conditions. We have throughout these papers held to the classification of Strasburger that the cytoplasm may be separated into two forms: kinoplasm and trophoplasm, which show certain structural peculiarities and are characterized by very different forms of activity. While it must be acknowledged that kinoplasm and trophoplasm are very similar in certain regions of the cell and at certain periods of the cell history, still the distinctions are in general clearly marked.

Kinoplasm is homogeneous in structure, either minutely granular or consisting of delicate fibrillæ composed of very small granules placed end to end. The homogeneous condition is characteristically shown in the three forms of plasma membranes which cytoplasm places between itself and external or internal surface contacts. The three membranes are: the outer plasma membrane, the nuclear membrane, and the vacuolar membranes. They are certainly closely related and probably identical in structure and appear to be the natural expression of protoplasm to contact with a fluid (water) medium. The fibrillar condition appears during mitosis and serves important functions in the mechanism (spindle) through which the chromosomes are distributed and in most of the higher plants determines the position of the cell wall that is generally formed with each nuclear division.

But the manifestations of kinoplasm during nuclear division and also in relation to cilia-bearing surfaces are exceedingly various and it is among these structures that our ignorance of relationships and modes of origin is deepest. These kinoplasmic structures have been described in various connections throughout this series of papers and especially in Sections I, II, and III, and need not be treated here. But the point which should be emphasized in this connection is the necessity of the close study of their simplest expressions in the lower regions of the thallophytes. The most varied forms of kinoplasm are in the thallophytes where asters, centrospheres, and centrosomes obtain and where ciliated cells, presumably with blepharoplasts, may occupy long periods of the life history. It is here that we must search for information that will bring order out of the confusion of our present accounts and insufficiency of knowledge. The most vital problems relating to kinoplasm concern the origin and the events of the simplest types of mitotic phenomena and the formation of cilia. We have a fairly clear understanding of the general features of mitosis in the groups above the thallophytes and their relation to the lower types and these will be briefly treated in the following portion of this section under the head: "Some Apparent Tendencies in the Evolution of Mitotic Phenomena." But the events of mitosis among the thallophytes are exceedingly various and difficult to understand and nothing is known of their origin or relation to the simpler conditions which must be present in the lowest regions of the Chlorophyceæ and in the Cyanophyceæ.

Trophoplasm comprises all of the cytoplasm included within the plasma membranes. While this region does not give rise to such highly differentiated cell organs as the kinoplasm, nevertheless some remarkably interesting structures are developed. Cœnocentra and Physodes are specialized structures of exceeding interest and our ignorance of the latter is truly remarkable. Nematocysts if trophoplasmic offer another attractive subject for investigation. In a sense, chromatophores and plastids may be considered trophoplasmic but their high grade of specialization and fixity as cell organs gives them a certain independence of other structures in the cell. Respecting the structure of the

groundwork of trophoplasm, whether fibrillar, granular, or presenting the structure of foam, botanical science has as yet furnished very little systematic study and this field of research is one of exceptional opportunity for the student of the plant cell.

The Cell Wall.— The cell wall may be treated from two points of view: either with respect to the strict chemistry of its organization and development or more largely for the biological and morphological features involved. The chemistry of the cell wall is an exceedingly complex subject which has developed a special literature of its own. In the substance termed cellulose we are not dealing with a single body but rather with a large group of closely related bodies. And besides the members of the cellulose group there may be present foreign substances so intimately associated with the carbohydrates as to resist very severe treatment. We cannot even touch this phase of the subject; a brief review of its complexities and problems is presented by Beer (:04) and there are further references in Section I of these "Studies."

There are, however, some biological features of the process of wall formation, the morphological and physiological aspects of the phenomena as they are related to protoplasm, which offer some exceedingly interesting problems especially among the thallophytes. It has long been a matter of dispute whether the cell wall is a secretion from the surface of a plasma membrane or is formed wholly or in part by the transformation of such a membrane.

It seems to be established now that substances of the cellulose groups are only formed in contact with plasma membranes, that is, they are not formed actually in the interior of protoplasm although they may appear to lie in such situations. Thus the material of the capillitium of the Myxomycetes which is of the same character as the chief substance in the exterior covering of the fructification, is laid down within vacuoles in the protoplasm, and is therefore in contact with the surface of vacuolar plasma membranes precisely as the outer covering lies in contact with the surface of the outer plasma membrane. The morphological relation of capillitium and outer covering to the surface of plasma membranes is therefore precisely the same.

And similarly the cross wall which takes the position of the cell plate at the end of mitosis is not developed from the transformation of a film of protoplasm but is laid down between two surfaces that separate to form a thin vacuole which later spreads to the edge of the cell and the wall is deposited between these two membranes which are almost in contact. There are a number of cases in which large strands or masses of protoplasm have been described as changing directly into cellulose but it is probable that these examples upon further study will exhibit the same relation of the cellulose substances to plasma membranes as in the typical cases of wall formation. There are many interesting examples of cellulose formation whose precise relation to the protoplasm has not yet been determined.

Respecting the exact method by which a cellulose wall is laid down by a plasma membrane there is very little real information. It is clear now that the cellulose is not a secretion from the plasma membrane comparable to a mineral shell. There is much evidence that protoplasm is actually sacrificed in the development of cellulose. There are numerous illustrations, as in the tracheids and other cells empty of protoplasm, where the final secondary thickenings are deposited as the protoplast grows smaller and eventually disappears, a large part of its substance evidently contributing to the deposits which are members of the cellulose group. But of course it cannot be supposed that the molecules of the proteids are changed directly into those of the carbohydrates. Nevertheless it does seem clear that the carbohydrates appear simultaneously with the disappearance of the proteids and occupy the position formerly held by the latter. It is probable that with the splitting up of the proteid molecule, carbohydrate material is formed which displaces the proteid substances. So in a broad sense the cellulose deposit actually does represent a transformation of a plasma membrane.

The evidence in general favors the view that the wall, lamellæ, and other deposits of cellulose only increase in amount when in actual contact with a plasma membrane. Some apparent exceptions to this principle are easily understood. Thus cell walls or portions of such may swell greatly and become much softer in consistency and perhaps even mucilaginous. There are no

reasons for regarding such transformations as an actual increase in the carbohydrate material for it is clear that the substance is a body with a greater amount of water in its organization than is present in the more usual forms of cellulose compounds. But there are some cases which are not so easily understood and perhaps the most widely known are the megaspore walls of certain species of Selaginella. These spores are remarkable for a differentiation of the spore wall in which the outer layer seems to be entirely separated from the inner by a space and yet is able to increase enormously in size and take on marked peculiarities of structure, but apparently without any relation to the protoplast. It may, however, be justly questioned whether the apparent space between the inner and outer wall is really a cavity and may not be filled with plastic material which holds the two walls in intimate organic relation to one another and to the protoplast. Miss Lyon has recently given this subject attention and announced her belief that the latter condition obtains. Her conclusions will be awaited with interest.

As regards the way in which a cell wall increases in size we are still limited to the two conceptions termed (1) growth by apposition and (2) growth by intussusception. The first method consists in the laying down of successive layers by the plasma membrane and results in a thickening of the cell wall. It is of course a comparatively simple process. Growth by intussusception is a stretching or expansion of the substance which seems to be greatly increased in quantity although the morphology of the structure remains the same. The current explanation outlined by Nägeli assumes that new molecules of carbohydrates are intercalated among the old. It seems more probable that the increase in bulk is due to some modification or rearrangement of existing molecules, involved, perhaps with an increase of material but not through the actual intercalation of new molecules of the same or original carbohydrates. The chemistry of the carbohydrates is so complex that great changes of form, bulk, or optical properties may be readily assumed which would quite change the appearance of a structure, without, however, necessitating the transportation of new carbohydrate substance to it directly.

There are many forms, particularly among the lower plants, where studies on the processes of wall formation are sure to throw much light on the fundamental problems which we have discussed. And a particularly interesting study might be made of the evolutionary history of the cell wall among the thallophytes and in the modifications introduced when plants pass from aquatic habits to aërial or terrestrial conditions. Our attention has been chiefly centered on the structure of the protoplast and the morphology and behavior of its parts. We are likely soon to give more study to the carbohydrate membranes and walls and this subject is likely to be very fruitful for investigation.

3. Some Apparent Tendencies in the Evolution of Mitotic Phenomena.

Our brief descriptions in Section II (*Amer. Nat.*, vol. 38, p. 431, June, 1904) of the various kinoplasmic structures developed during mitosis in different groups of plants brings up the problem in their relationships to one another, *i. e.*, the evolutionary tendencies in the differentiation of mitotic phenomena. We have seen that the thallophytes present an especially diverse assortment of kinoplasmic structures associated with the spindle and its method of development. The spindle fibers, whether formed within the nuclear membrane (intranuclear) or arising from without (extranuclear), are associated with centrosomes or centrospheres to form asters in a number of well known types as Stypocaulon, Dictyota, Fucus, Corallina, certain diatoms, the ascus, and the basidium. Centrospheres are found in certain phases of the life history of liverworts as in the germinating spore of Pellia. A second type of kinoplasmic structure resembling in certain features the aster but with some fundamental differences has been termed the polar cap. The polar cap is an ill defined region of kinoplasm, generally larger than a centrosphere and without clear boundaries, which forms a region for the insertion of spindle fibers. Polar caps are well illustrated in the mitoses of vegetative tissues and meristematic regions, especially among the higher plants (pteridophytes and sperma-

tophytes). They sometimes approach the centrosphere very closely in their morphology.

The third and highest type of spindle formation in plants is that illustrated in the mitoses within the spore mother-cell which were given special treatment in Section III (*Amer. Nat.*, vol. 38, p. 725, October, 1904). In this remarkable cell the spindles develop from a mesh of independent fibrillæ which at prophase more or less completely surround the nucleus. The poles of the spindle arise by the grouping of cones of fibrillæ so that a single axis is finally established but without any kinoplasmic centers at the poles. This type of spindle formation which may be termed the free fibrillar type is one of the most interesting cytological peculiarities of plants. It has been found in all types whose sporophytic phase terminates its history with a spore mother-cell, although the accounts in the Hepaticæ are not in full accord.

Is it possible to connect the various types of spindle formation with one another and to establish any evolutionary tendencies in the processes involved; and have the different manifestations of kinoplasm such as centrosomes, centrospheres, polar caps, free fibrillar condition, and the mysterious structure called the blepharoplast any genetic relation to one another? The confusion is so great among the thallophytes that the author sees little hope at present of establishing clearly any relationships between the types of centrospheres and centrosomes with their systems of radiations (asters) and we must patiently wait for more information. And respecting the origin of these structures from the simpler types of mitosis we are absolutely in the dark. But the relation which polar caps and the free fibrillar type of spindle formation bear to centrospheres is less perplexing and it seems possible to define certain common features among these structures which hold them together with a degree of unity in their relations to mitosis. That phase of the subject will be considered in this treatment. The Hepaticæ as a group occupy an interesting position with respect to the character of mitotic phenomena at various periods of ontogeny, between conditions in the pteridophytes, which are obviously similar to the spermatophytes, and conditions in the thallophytes. This was brought

out by the work of Farmer whose accounts of centrosomes and centrospheres in the germinating spores of Pellia and within the spore mother-cell of various liverworts, together with his account of a "quadripolar spindle" made it evident that the group offered some very interesting cytological problems. They led the author to the study Anthoceros (Davis, '99) and Pellia (Davis, :01), investigations which have been followed by Van Hook (:00) on Marchantia and Anthoceros, Moore (:03) on Pallavicinia, Chamberlain (:03) and Grégoire and Berghs (:04) on Pellia, while Ikeno (:03) has studied the processes of spermatogenesis in Marchantia.

My studies on sporogenesis in Anthoceros and Pellia led me to conclude that the processes of spindle formation did not differ in any essentials from those in the pteridophytes and spermatophytes. There are present two successive mitoses and the spindles are formed from a surrounding mesh of fibrillæ developed from the kinoplasm associated with the nuclear membrane and without achromatic centers (centrospheres or centrosomes). They exhibit clearly the free fibrillar type of spindle formation although in somewhat simpler form than in the pteridophytes and spermatophytes. The poles of the spindles generally end bluntly in areas of granular kinoplasm but these seem to me too indefinite in form to deserve the designation of centrospheres and such granular inclusions as may be present are too variable in number and position to be termed centrosomes. There is clearly present in Pellia during the prophase of the first mitosis a four-rayed achromatic structure which is later replaced by a typical bipolar spindle. This four-rayed kinoplasmic structure is evidently the same as Farmer's "quadripolar spindle" which he described as associated with a simultaneous distribution of the chromatin in Pallavicinia to form at once four daughter nuclei. I was led to doubt this account and to suggest that the "quadripolar spindle" might prove to be simply a phenomenon of prophase associated with the peculiar four-lobed structure of the spore mother-cell in the Jungermanniales. I stated my belief that the distribution of the chromosomes during sporogenesis in all liverworts would be found to take place through two successive mitoses after the usual manner. Moore (:03)

has recently studied an American species of Pallavicinia and has failed to confirm Farmer's conclusions. He found the four-rayed figure, which Farmer terms a "quadripolar spindle," a conspicuous feature of the first mitosis here as in Pellia but there was no indication of a simultaneous distribution of quadrupled chromosomes to form four daughter nuclei as reported by Farmer. The four-rayed figure was merely preliminary to the first mitosis whose spindle at metaphase was bipolar and the first mitosis was followed shortly by a second, so that Pallavicinia offers no exception to the essential features of sporogenesis as known in all groups above the thallophytes.

Farmer (*Bot. Gaz.*, vol. 37, p. 63, 1904) has taken exception to the restriction of the term spindle by Moore and myself to the structure found at metaphase and holds that the four-rayed structure is a part of the spindle apparatus. In this discussion he appears to avoid the issue, which is not the broader or narrower application of the term spindle, a mere matter of usage, but concerns the fundamental character of the mitoses during sporogenesis whether they are two in number and successive in all forms or whether Pallavicinia presents an extraordinary exception in a distribution of the chromatin to form four daughter nuclei simultaneously in the spore mother-cell. Farmer (: 05) has recently reaffirmed his view that the poles of the four-rayed figure in Aneura and presumably in other Jungermanniales are occupied by centrospheres and that sometimes a central body (centrosomes) may be distinguished in each. This statement involves again a matter of usage in which I should differ from Farmer for my studies and those of Moore do not seem to me to justify the application of these terms to regions of kinoplasm whose form is so ill defined and history so transient within the cell.

These disputed points which were also discussed in Section III (*Amer. Nat.*, vol. 38, pp. 727–732, October, 1904) are of importance in relation to the mitotic phenomena in other periods of the life history of liverworts which will now be considered. It may be stated, however, that other investigators who have studied the processes of sporogenesis in the liverworts (Van Hook, : 00; Chamberlain, : 03; Grégoire and Berghs, : 04) sup-

port my general program of sporogenesis with the free fibrillar type of spindle formation. There seems to be little question but that centrospheres are present and conspicuous in the early mitoses within the spore of Pellia. They have been especially studied by Farmer and Reeves ('94), Davis (:01), Chamberlain (:03), and Grégoire and Berghs (:04). All of these authors have agreed that asters are clearly defined in the early mitoses within the spore and most of them have termed the region of kinoplasm in the center of the aster a centrosphere. The structures are less prominent in the third mitosis and are perhaps replaced in later periods of the gametophyte history by kinoplasmic polar caps. Polar caps are characteristic of the mitoses in the seta of Pellia (Davis, :01). However, Van Hook has described centrospheres with radiations at the poles of the spindles of the archegoniophores of Marchantia, whose centers sometimes contained centrosomes, and it is possible that the centrosphere runs through a considerable period in the life history of liverworts. There is complete agreement that the centrospheres when present arise *de novo* and independently of one another during the prophase of mitosis and that they disappear at telophase. Ikeno has, however, described centrosomes during the mitoses within the antheridium which are said to divide and pass to opposite sides of the nucleus where they become the poles of the spindles. They cannot be found after the mitosis is completed, but are described as formed *de novo* in the interior of the nucleus and thrust through the nuclear membrane into the cytoplasm previous to each mitosis. After the final division in the antheridium, the centrosome remains to function as a blepharoplast.

Thus we see that the liverworts present during their life history an almost complete range of kinoplasmic structures associated with the nuclear divisions from centrosomes and centrospheres to polar caps and that type of spindle formation characterized by free fibrillæ gathered into cones but entirely independent of definitely organized centers. There is also present the blepharoplast. I emphasized this range of kinoplasmic structure in my paper on Pellia and it seemed to me one of the most interesting features of the liverworts. In this paper

(Davis, :01, p. 171) are outlined the changes in form which kinoplasm may assume in the mitoses of the liverworts upon which is based a theory of a cycle through which kinoplasm may run in the history of a cell. On this theory, centrosphere, polar cap, and the free fibrillar condition are all secondary developments from a primal finely granular kinoplasm which is the only form of kinoplasm that is in any sense permanent in the cell. This finely granular kinoplasm is always present in characteristic form in the plasma membranes of the cell. The substance of centrospheres, polar caps, and fibrillæ arises from accumulations of granular kinoplasm during prophase and these structures return to the same undifferentiated granular kinoplasm at the end of mitosis or become lost in the general cytoplasm of the cell.

The cycle is from an undifferentiated finely granular kinoplasm through certain specialized conditions either wholly or in part fibrillar in structure back to the granular state. The centrosphere and polar cap are regions from which fibrillæ develop at least in part and to which they may remain attached as to an anchorage. The polar cap is a less clearly differentiated kinoplasmic center than the centrosphere but does not differ from it in the essentials of its organization. It seems to me that the two structures are very closely related in the liverworts and that in this group we may readily conceive the polar cap as derived from the centrosphere. The free fibrillar type of spindle formation is a step farther in the direction of such a distribution of the kinoplasm that no very positive centers for the development of the spindles may be distinguished. The four-rayed structure (quadripolar spindle) so characteristic of the spore mother-cell in the Jungermanniales represents a group of four temporary centers for the formation of fibrillæ and there is clearly a gathering of kinoplasm at these points but the regions are so vague in outline as hardly to justify the designation of centrospheres. From the fibrillar state, kinoplasm returns to the finely granular condition by the contraction of the fibers which thus contribute their substance to some common area. The area may lie around the chromosomes of the daughter nuclei where it becomes later in part at least a nuclear mem-

brane. Or the area may be a cell plate whose halves on division finally merge with outer plasma membranes of the cells. The spindle fibers which cut out the spore areas in the ascus form the basis of a plasma membrane. Thus the fate of all kinoplasmic fibrillæ seems to be a final return to the undifferentiated, finely granular condition so characteristic of plasma membranes which according to this theory is the condition from which they arose.

Thus I believe the liverworts present rather striking evidence of a relationship between the centrosphere, polar cap, and the free fibrillar condition of spindle formation and establish an evolutionary tendency from the first two types of kinoplasmic differentiation towards the latter. The free fibrillar type of spindle formation is found in a very simple form in this group, sometimes with temporary centers, as in the four-rayed figure (quadripolar spindle) of prophase, whose poles have accumulations of kinoplasm in the position of centrospheres. The polar caps are likely to prove a much simplified type of centrosphere whose kinoplasm is no longer gathered to form conspicuous spherical centers. With respect to the problem of the homologies and nature of the blepharoplast, the liverworts furnish as yet no material assistance and this structure stands at present as one of the most interesting puzzles of plant cytology. As stated in the beginning, the variety of centrosomes and centrospheres with and without radiations in various types of the thallophytes seems to me too confusing to promise an understanding of their relationships at present.

Grégoire and Berghs (:04) have interpreted the structure of the mitotic figure in the germinating spore of Pellia in a very different manner from the accounts of Farmer, Chamberlain, and myself. They consider the asters to arise through a rearrangement of the cytoplasmic network around the nucleus. They affirm that there are no true centrospheres nor any accumulations of granular kinoplasm to constitute the centers of origin for the spindle fibers or the radiations around the poles of the spindle. The centers of the asters ("vésicules polaires") are said to have a vesicular structure and neither they nor the nucleus contributes to the building up of the spindle which is

developed entirely out of the cytoplasmic network. The authors are unable to distinguish a kinoplasm distinct from the general network of the cell. These are vital points of difference which are fundamental to the understanding of mitotic phenomena and rest of course on matters of fact. The chief points at issue concern the structure and development of the asters and the nature of the material at their centers. My own studies and those of Farmer and Chamberlain have convinced me that there is an accumulation of substance (kinoplasm) in the centers of the asters and polar caps to such an amount that it must be regarded as a definite structure in the cell and its morphology and relations to the spindle have certainly justified us in considering it as similar to the centrosphere of the thallophytes.

4. THE ESSENTIAL STRUCTURES IN THE PLANT CELL AND THEIR BEHAVIOR IN ONTOGENY.

The cell is composed of a series of osmotic membranes between which are included a number of protoplasmic structures whose morphology and minute organization is various. They are: the outer plasma, the vacuolar, and the nuclear membranes. Each of these sustains a relation to some fluid which bathes its surface. The fluid nature of the nuclear sap and cell sap is obvious but the outer plasma membrane is also against a moist surface since the cell walls of tissues are normally saturated with water. The structure of the plasma membranes is apparently the same. They consist of the homogeneous finely granular protoplasm that is designated kinoplasm. The protoplasmic structures included within the plasma membranes may be grouped as cytoplasmic and nuclear. The greater part of the cytoplasm, including that which is termed trophoplasm, has an organization peculiar to itself and very different from that of the plasma membranes. This structure has been described as alveolar or of the nature of foam and sometimes fibrillar and with various large granular inclusions. The cytoplasm also contains the characteristic organs termed plastids. The conspicuous structures of the nucleus are: the chromatic elements

appearing as chromosomes during mitosis and the nucleoli. These structures are so easily recognized and play such important parts in the events of nuclear division that they command attention at once as the essential elements in the nucleus. The nucleus may also contain other material such as linin which, however, does not seem to have a fixed form or behavior in the cell. Finally there are certain kinoplasmic structures, as centrosomes, centrospheres, and blepharoplasts, whose behavior throughout cell history has been much discussed. We shall now consider the most important of these structures, those which seem essential to the cell in ontogeny.

The outer plasma membrane naturally retains its morphological entity throughout all cell divisions with such slight changes as when new parts are intercalated into its area through the vacuoles that are utilized in the segmentation of protoplasm. Vacuolar membranes are constantly shifting and cannot be followed during cell division excepting in such cells as have one large central vacuole (the tonoplast of De Vries). Such a central vacuole is much more characteristic of old cells and tissues than of young or embryonic regions. There is certainly no reason to suppose that it has organic existence through any very extended period of the life history. The nuclear membrane becomes lost during the prophase of mitosis and there is much evidence that its kinoplasm contributes in some cases to the formation of spindle fibers. Thus the nuclear membrane disappears as a structure in the cell during mitosis and new vacuoles are formed around the assemblages of daughter chromosomes during telophase, leading of course to the formation of fresh nuclear membranes at their surface of contact with the surrounding cytoplasm.

There is perhaps no region of the cell protoplast that presents such different appearances through long periods of the cell history as the trophoplasm. This is largely due to the varying character of the inclusions which are not in themselves protoplasmic but which give a mixed structure to the trophoplasmic regions of the cell. The inclusions may be carbohydrate or proteid bodies held within spaces in the trophoplasmic groundwork or they may be globules of oil or fatty substances. These

inclusions occupy small spaces in the trophoplasm which are essentially vacuoles. There is also a class of granular inclusions of a proteid nature which probably represent material in very close organic relation to the substance of protoplasm. Trophoplasm does not then have so clearly defined a type of structure as do the other regions of the protoplast but it is hardly probable that its essential nature changes very materially throughout the life history. The organization of trophoplasm is itself a matter of dispute but the prevailing views favor an alveolar or foam structure with a fibrous character at times somewhat resembling the texture of sponge.

Ever since the classical investigations of Schimper upon the plastid it has generally been held that these structures are permanent organs of the cell, reproducing by fission, and carried along from one cell generation to the next with as much permanence as the nucleus. Schimper discovered plastids in the oöspheres of certain spermatophytes and in a variety of embryonic tissues and concluded that the structures passed from parents to offspring as leucoplasts when no trace of color could be found in the reproductive cells or embryonic tissues. There has been, however, no systematic study of the plastid throughout the life history of higher plants and in most of the green thallophytes there are reproductive phases, such as resting spores, where we have no knowledge of the structure or distribution of the chromatophores in the cell. It is very important that the plastid be investigated with the same degree of attention which has been given to the nucleus, and that it be followed through all periods of the life history in forms where the color becomes greatly modified or is absent in the reproductive cells and embryonic (meristematic) regions of the plant. Anyone who has studied the embryonic tissues of plants will realize the difficulties of the investigation which will probably involve the development of methods of technique, especially of staining, somewhat different from those generally employed in cell studies.

We may now consider the elements in the nucleus and their behavior during ontogeny. This is one of the most interesting subjects in cell studies, for the importance of the chromosomes

and chromosome history in relation to problems of development, heredity, hybridization, and variation is clearly understood, and these subjects have already been treated in Section V, "Cell Activities at Critical Periods of Ontogeny in Plants." Also some recent papers on the nucleolus of which Wager's (: 04) is the most comprehensive, have brought this structure into very close relation with the chromatin content of the nucleus, and the nucleolus must now be considered in any treatment of the chromosomes. The problems hinge on what is termed the individuality of the chromosome, which is the question whether or not the chromosome is a structural entity maintaining its independence completely through each and all of the cell divisions in a life history. There is also involved the view that the chromosomes have come down from a line of ancestral structures, reproducing by fission in every mitosis throughout the history of the race.

There are two extremes in the views on this exceedingly interesting conception and also an intermediate position. The one extreme has recently been set forth by Boveri (: 04) in a very clear statement. This view regards the chromosomes as structural entities, possibly elementary organisms, which maintain an organic individuality and independent existence in the cell. They are further regarded as in their typical form when present as rods or short filaments during mitosis. Their behavior in the resting nucleus is one of great metabolic activity which affects their morphology for the time being.

Those who are inclined to doubt the individuality of the chromosomes and to hold off from a full acceptance of the theory, base their attitude on the extreme difficulty or perhaps impossibility of following the chromosomes as entities through the resting nucleus from one mitosis to another. These difficulties are well known to those who have studied chromosomes even in nuclei which are most favorable for the investigation of their morphology. The chromosomes which enter the daughter nuclei from a mitosis generally lose their form and the chromatin becomes so distributed on a linin network or in a nucleolar structure that the outlines of the original structures become quite lost. Mottier (: 03) in his recent studies on the spore

mother-cell of certain angiosperms has emphasized these points and Grégoire and Wygaerts (: 03) have also shown the difficulties of following the chromosomes in the resting nuclei of the root tip and spore mother-cell of Trillium, stating that the structures become resolved into an alveolar network.

On the other hand Rosenberg (: 04) claims that the chromosomes may be clearly recognized in the resting nuclei of some forms and cites *Capsella bursa-pastoris* as a particularly good illustration. In this plant the chromosomes are described as small granular bodies scattered throughout the nucleus in fixed number at various stages of ontogeny. Thus there are 16 in cells of the gametophyte and 32 in those of the sporophyte while 48 of these bodies were counted in the nuclei of the endosperm as would be expected if these nuclei are descendants of a triple fusion in the embryo-sac. Similar conditions are reported in other forms and there is considerable evidence giving weight to the view that chromosomes may be actually followed through all periods of the nuclear history in some favorable types.

Apart from the actual demonstration of the chromosomes in the resting nuclei and their recognition as structural entities through successive cell divisions there is much general evidence in support of the theory of the individuality of the chromosomes. This evidence lies in the nuclear fusions of fertilization and the mitoses of processes of segmentation that follow where the chromosomes are known to remain separate and have been distinguished as maternal and paternal. Also, as we have seen from the discussions of reduction phenomena at sporogenesis and the behavior of the chromosomes in hybridization, there are good reasons for believing that maternal and paternal chromosomes remain separate all through the sporophyte generation and are distributed to the offspring during sporogenesis. The importance of these events in the minds of all investigators has rested very largely on the behavior of the chromosomes and has led to the very general assumption that they must stand for units of organization and may be counted as constant factors in the problems of heredity. It is not necessary to adopt Boveri's extreme views to hold still the theory of the individuality of the chromosomes. Nor is it necessary to assume that the structures

have a distinct organization which holds throughout the life history. The form of the chromosomes certainly does change with different periods of the cell's history especially within the resting nucleus and yet the centers of chromosome activity may always be present to organize the chromatin into a new set of elements for the next mitosis. It is perhaps difficult to believe that the chromatin granules (chromomeres) find their way back to the same chromosome with the prophase of each mitosis but the existence of chromosome centers may be readily conceived within the resting nucleus which would hold the number of chromosomes true to the cell's history.

With respect to the nucleolus there is abundant evidence that the structure is not a permanent organ of the cell. When containing chromatin, the nucleolus is found in its characteristic globular state only during the resting condition of the nucleus. Its chromatic substance passes into the chromosomes at prophase of mitosis and the nucleolus generally disappears before metaphase. Or if any substance is left after the chromosomes are formed the remaining structure either gradually dissolves or is thrust forth bodily into the cytoplasm surrounding the mitotic figure where it disappears sooner or later. The nucleolus in higher types of mitosis never divides to pass on with the chromosomes to the daughter nuclei, but such a history is reported in the yeast cell. If the nucleolus has any function in heredity, as has been claimed (Dixon, '99), such function must relate to the chromosomes which contribute to its substance or derive material from it. Besides the nucleoli which are composed wholly or largely of chromatin, there are also those which seem to have little if any relation to the chromosomes. Such are well known in the spore mother-cells of higher plants and no investigator has been able to connect these with the formation of chromosomes as Wager (: 04) has been able to do in the root tip. It was upon nucleoli of this class that Strasburger founded his theory that the structure was a mass of reserve material utilized by the kinoplasm during mitosis in the process of spindle formation. Such nucleoli generally fade away during the prophase of mitosis and either entirely disappear or the remaining substance is thrust out into the cytoplasm where it may some-

times be recognized as deeply staining globules (the so called extranuclear nucleoli).

There is left for our consideration that group of kinoplasmic structures termed centrosomes, centrospheres, and blepharoplasts which, when accompanied by radiations, are called asters. Some authors regard these structures as homologous and believe them to be present in one form or another as permanent organs of the cell in certain types (see discussion of Ikeno, :04). Against this view stand the well established facts of an increasing list of forms, both animals and plants, in which these structures unquestionably arise *de novo* at certain periods in the cell's history. To the author this evidence seems insurmountable and he cannot believe that the aster is in itself a permanent organ of the cell. We shall not take up the subjects of relationships here for such discussions have proved of little profit except in special cases where the various types of structure are found in closely related forms or in the same life history, and these have scarcely been studied at all. We know so little about the relationships in the thallophytes, where relationships must be sought if present at all, that a satisfactory treatment of the subject is hardly possible at present. One point seems to have escaped attention in the writings of those who have discussed the centrosome problem. The active elements of the asters are not the central structures (centrosomes, centrospheres, or blepharoplasts) but the fibrillæ which play such important parts as spindle fibers or cilia. This fibrillar condition of kinoplasm has a fixed place in the cycle of cell division appearing with each mitosis and at the time of cilia formation, but the fibrillæ are not permanent structures of the cell. There is some evidence that the centrosomes, centrospheres, and blepharoplasts are merely regions for the development and attachment of these fibrillæ and as such may stand as the morphological expression of fibrillæ-forming dynamic centers rather than as organs which actually induce the development of fibrillæ.

5. The Balance of Nuclear and Cytoplasmic Activities in the Plant Cell.

Two regions of the cell are sharply distinguished from one another with respect to both morphology and physiology. They are the nucleus and the cytoplasm. The nucleus soon dies if isolated from cytoplasm and the latter, lacking a nucleus, cannot be kept alive indefinitely unless it be in organic connection with a nucleated mass of protoplasm. The necessary connection may be only through delicate strands, as was established by Townsend ('97), and also seems to be illustrated in the instances of intercellular protoplasm which Michniewicz (:04) reports are connected by delicate fibrillæ (plasmodesmen) with neighboring cells. Some very interesting adjustments of the nucleus and cytoplasm to one another have been reported in a series of investigations of Gerassimow beginning in 1890. His most recent papers of the past year (Gerassimow, :04a, :04b) present a general summary of his studies and constitute a very important contribution to the subject. They will furnish much of the material for this discussion.

Gerassimow has found that the cells of Spirogyra and other members of the Conjugales offer admirable material for the study of the relations between the nucleus and cytoplasm, and throw important light on the functions, physiological activities, and interdependence of both structures. By subjecting filaments of Spirogyra during cell division to a temperature of 0° C. or treating them for a short time to the anæsthetic influence of ether, chloroform, or chloral hydrate it is possible to arrest the processes of mitosis at different stages with the result that the protoplasm may become variously distributed in the daughter cells. (1) A daughter cell may be formed lacking a nucleus but containing a portion of the divided chromatophore in a peripheral layer of cytoplasm. (2) A single cell may contain the two daughter nuclei either separated from one another or more or less intimately associated and perhaps wholly fused depending upon how far the processes of mitosis have progressed before the cells have been subjected to the shock of the

experiment. (3) Binucleate cells may continue their growth with subsequent mitoses which when treated as before may give daughter cells with three nuclei and one nucleus respectively or with two each or indeed a cell containing four nuclei. Furthermore these nuclei may fuse with one another to give structures with a greatly increased chromatin content. (4) In place of the non-nucleated cells there may be formed chambers containing cytoplasm and chromatophores, but without nuclei, which remain in open communication with the nucleated companion protoplast because the cell wall is not formed entirely across the mother-cell.

Gerassimow has made some extended observations on these various types of cells, and presents his results in many elaborate tables and diagrams. We can only give an outline of his conclusions. (1) Cells which come to contain unusually large nuclei through the suppression of mitosis or by the reuniting of partially divided daughter nuclei increase proportionally in size and their further cell division is postponed. The nuclei of such cells have of course the peculiarity of an increased amount of chromatin content. The large nuclei may later fragment into two or more structures which separate and generally come to lie at a distance from one another in the cytoplasm. The fragments finally lose their powers of reproduction and exhibit marked evidence of degeneration. (2) Cells which lack nuclei may form starch in the usual manner in the presence of light and exhibit for a short time a weaker general growth than normal nucleated cells. The power to develop a gelatinous sheath also becomes markedly weakened. Finally there result a decrease in the volume of the cell, a fading of the chromatophore, and conditions which lead to eventual death. (3) Chambers which lack nuclei but are in protoplasmic union with nucleated cells may be contrasted sharply with the non-nucleated cells. They exhibit a much stronger growth for a longer time and with a greater power to form starch, although not so marked as in the nucleated cells, and the chromatophores retain their color. There is also a conspicuous development of the gelatinous sheath.

Haberlandt, Klebs, Pfeffer, Strasburger, and others have dis-

cussed the relations of the nucleus to the surrounding protoplasm with respect to both dynamics and morphology. Klebs ('88) indeed anticipated some of the work of Gerassimow, studying the non-nucleated cells of Zygnema and Spirogyra and noting the ability of their chromatophores to form starch in considerable quantities but the inability of the protoplast to add to the cell wall. Klebs was able to keep these non-nucleated cells alive in a sugar solution for from four to six weeks. But for the most part the discussions of the balance of nuclear and cytoplasmic activities in the plant cell have been very general in character.

Some principles have been, however, widely held for several years and may be summarized. The necessity of the nucleus to the life of the cytoplasm has been clearly understood but the studies of Klebs and Gerassimow indicate that the nucleus is not directly concerned with the process of photosynthesis which apparently may go on in non-nucleated cells as long as the cytoplasm retains a certain degree of vitality. A non-nucleated cell may enlarge slightly but it is not probable that the amount of protoplasm is increased. An especially interesting feature of non-nucleated cells is the inability of the outer plasma membrane to form cellulose walls or outer membranes. But the very interesting studies of Townsend ('97) have shown that this power may be retained provided the non-nucleated mass of protoplasm is connected by delicate cytoplasmic fibrils with a nucleated mass. It thus seems clear that the membrane-forming possibilities of the outer plasma membrane are absolutely dependent upon dynamic relations with the nucleus. While the chromatophore may carry on the processes of photosynthesis independently of the nucleus, nevertheless the general health of the cell requires the activities of the latter so that the nucleus becomes necessary to any extended photosynthetic work.

It has frequently been stated that the size of the nucleus is directly proportionate to the amount of cytoplasm in the cell. There are many favorable illustrations of this statement, as the extraordinarily large eggs of the gymnosperms, especially the cycads, whose nuclei are by far the largest in the plant kingdom. And in general an increase in the amount of cytoplasm is accom-

panied either by a marked enlargement of the nucleus with a corresponding increase in the chromatin content or by mitoses which distribute to the cytoplasm a greater number of nuclei whose sum total of material is very much greater than before. Conversely a sudden increase in nuclear material through nuclear fusions either sexual or asexual is followed almost immediately by general cell growth and increase in the amount of cytoplasm. However, such fixed growth relations between nucleus and cytoplasm can hardly be an established physiological law for certain highly specialized sperms have an insignificant amount of cytoplasm proportionately to the chromatin that is contained within the gamete nucleus. It is evident that the interrelations of the nucleus and the cytoplasm are so intimate that the growth activities of the one must benefit the other, but that this principle can be formulated in definite mathematical ratios seems improbable.

The dependence of the nuclei upon favorable situations in the cytoplasm is clearly shown in cells when a partial or general nuclear degeneration takes place. Thus during the processes of oögenesis in the Peronosporales, Saprolegniales, and in Vaucheria there is present a period when the most of the numerous nuclei within the oögonia begin to break down and finally become disorganized. The causes of the nuclear degeneration are not entirely clear but apparently the organ is unable to supply all of the nuclei in their respective situations in the cytoplasm with the conditions necessary for their life. There is consequently a sort of struggle for existence among these numerous nuclei and only those that are favorably placed in the cell are able to survive. In all forms the surviving nuclei occupy a situation in the center of the masses of protoplasm which are to become the eggs and those that break down are at or near the periphery of the cell. In several genera (*e.g.*, Albugo, Peronospora, Pythium, Sclerospora, Saprolegnia, and Achlya) the surviving nuclei seem to owe their good fortune to a very close association with the cytoplasmic structure termed the cœnocentrum. The cœnocentrum is a clearly differentiated region of the cytoplasm and is probably the morphological expression of a dynamic center in the eggs of these fungi. Stevens' ('99, :01)

studies on Albugo showed that the cœnocentra exert a chemotactic influence upon the nuclei in their vicinity, drawing them towards the mass of granular material in this favored region of the cell, and it is clear that they are greatly benefited in this situation since they increase in size while the nuclei at the periphery break done. This subject is discussed in detail in my paper on Saprolegnia (Davis, : 03, pp. 240–243) a form which also illustrates exceptionally well the same principles of a survival of certain nuclei among many which degenerate, because of their favorable position in the central region of the eggs in close proximity to cœnocentra. There are then undoubtedly regions of the cell more favorable for the nutrition of nuclei than others and the positions of these may be marked by morphological characters as illustrated in the cœnocentra. That similar dynamic centers may also be present when there is little morphological evidence of their existence is indicated in the processes of oögenesis in Vaucheria (Davis, : 04) which exhibits the same principles of extensive nuclear degeneration as are found in the Peronosporales and Saprolegniales and the survival of a single nucleus in the oögonium, apparently because it comes to lie in a mass of granular cytoplasm near the center of the oögonium.

LITERATURE CITED IN SECTION VI, "THE PLANT CELL."

BARKER.
:01. A Conjugating Yeast. *Phil. Trans. Roy. Soc. London*, vol. 194, p. 467.

BEER.
:04. The Present Position of Cell-wall Research. *New Phytologist*, vol. 3, p. 159.

BOVERI.
:04. Ergebnisse über die Konstitution der chromatischen Substanz des Zellkerns. Jena, 1904.

BÜTSCHLI.
'90. Ueber den Bau der Bakterien und verwandter Organismen. Leipzig, 1890.

BUTSCHLI.
'96. Weitere Ausführungen über den Bau der Cyanophyceen und Bakterien. Leipzig, 1896.

BÜTSCHLI.
:02. Bemerkungen über Cyanophyceen und Bakteriaceen. *Arch. f. Protistenkunde*, vol. 1, p. 41.

CHAMBERLAIN.
:03. Mitoses in Pellia. *Bot. Gaz.*, vol. 36, p. 29.

DAVIS.
'98. Kerntheilung in der Tetrasporenmutterzelle bei *Corallina officinalis* L., var. *mediterranea*. *Ber. d. deut. bot. Gesellsch.*, vol. 16, p. 266.

DAVIS.
'99. The Spore Mother-cell of Anthoceros. *Bot. Gaz.*, vol. 28, p. 89.

DAVIS.
:01. Nuclear Studies on Pellia. *Annals of Bot.*, vol. 15, p. 147.

DAVIS.
:03. Oögenesis in Saprolegnia. *Bot. Gaz.*, vol. 35, pp. 233, 320.

DAVIS.
:04. Oögenesis in Vaucheria. *Bot. Gaz.*, vol. 28, p. 81.

DIXON.
'99. The Possible Function of the Nucleus in Heredity. *Annals of Bot.*, vol. 8, p. 269.

FARMER AND MOORE.
:05. On the Maiotic Phase (Reduction Division) in Animals and Plants. *Quart. Jour. Micr. Sci.*, vol. 48, p. 489.

FARMER AND REEVES.
'94. On the Occurrence of Centrospheres in *Pellia epiphylla* Nees. *Annals of Bot.*, vol. 8, p. 219.

FEINBERG.
:00. Ueber den Bau der Bakterien. *Centralb. f. Bak.*, pt. 1, vol. 27, p. 417.

FEINBERG.
:02. Ueber den Bau der Hefezellen und über ihre Unterscheidung von einzelligen thierischen Organismen. *Ber. d. deut. bot. Gesellsch.*, vol. 24, p. 567.

FISCHER.
'97. Untersuchungen über den Bau der Cyanophyceen und Bakterien. Jena, 1897.

FISCHER.
'99. Fixirung, Färbung, und Bau des Protoplasmas. Jena, 1899.

GERASSIMOW.
:04a. Zur Physiologie der Zelle. *Bull. Soc. Imp. Nat. Moscou*, no. 1.

GERASSIMOW.
:04b. Ueber die Grösse des Zellkerns. *Beih. z. Bot. Centralb.*, vol. 18, p. 45.

GOLENKIN.
'99. Ueber die Befruchtung bei *Sphæroplea annulina* und über die Structur der Zellkerne bei einigen grünen Algen. *Bull. Soc. Imp. Nat. Moscou*, p. 343.

GRÉGOIRE AND BERGHS.
:04. La figure achromatique dans le *Pellia epiphylla*. *La Cellule*, vol. 21, p. 193.

GRÉGOIRE AND WYGAERTS.
:03. La reconstitution du noyau et la formation des chromosomes dans les cinèses somatiques. 1. Racines de *Trillium grandiflorum* et telophase homœotypique dans *Trillium cernuum*. *La Cellule*, vol. 21, p. 7.

GUILLIERMOND.
:03. Recherches cytologiques sur les levures. *Rev. Gén. Bot.*, vol. 15, pp. 49, 104, 166.

GUILLIERMOND.
:04. Sur le noyau de la levure. *Ann. Mycol.*, vol. 2, p. 184.

HEGLER.
:01. Untersuchungen über die Organisation der Phycochromaceenzelle. *Jahrb. f. wissensch. Bot.*, vol. 36, p. 229.

HIERONYMUS.
'92. Beiträge zur Morphologie und Biologie der Algen. *Cohn's Beitr. z. Biol. d. Pflan.*, vol. 5, p. 461.

HIRSHBRUCH.
:02. Die Fortpflanzung der Hefezelle. *Centralb. f. Bak.*, pt. 2, vol. 9, pp. 465, 513, 737.

IKENO.
:03. Beiträge zur Kenntniss der pflanzlichen Spermatogenese; die

Spermatogenese von *Marchantia polymorpha.* *Beih. z. Bot. Centralb.*, vol. 15, p. 65.

IKENO.

:04. Blepharoplasten im Pflanzenreich. *Biol. Centralb.*, vol. 24, p. 211.

JANSSENS.

:03. À propos du noyau de la levure. *La Cellule*, vol. 20, p. 337.

JANSSENS AND LEBLANC.

'98. Recherches cytologiques sur la cellule de levure. *La Cellule*, vol. 14, p. 203.

KOHL.

:03. Ueber die Organisation und Physiologie der Cyanophyceenzelle und die mitotische Teilung ihres Kernes. Jena, 1903.

KLEBS.

'88. Beiträge zur Physiologie der Pflanzenzelle. *Unters. a. d. bot. Inst. z. Tüb.*, vol. 2, p. 489.

LAWSON.

:03. On the Relationship of the Nuclear Membrane to the Protoplast. *Bot. Gaz.*, vol. 35, p. 305.

MACALLUM.

'99. On the Cytology of Non-nucleated Organisms. *Trans. Can. Inst.*, vol. 6, p. 39.

MANO.

:04. Nucléole et chromosomes dans le méristème radiculaire de *Solanum tuberosum* et *Phaseolus vulgaris.* *La Cellule*, vol. 22, p. 57.

MARPMANN.

:02. Ueber Hefen und über den Zellkern bei Saccharomyceten und Bakterien. *Centralb. f. Bak.*, pt. 2, vol. 9, p. 357.

MASSART.

:02. Sur le protoplasme des Schizophytes. *Recueil d. l'Inst. Bot. Bruxelles*, vol. 5, p. 251.

MERRIMAN.

:04. Vegetative Cell Division in Allium. *Bot. Gaz.*, vol. 37, p. 178.

MICHNIEWICZ.

:04. Ueber Plasmodesmen in den Kotyledonen von Lupinus-arten und ihre Beziehung zum interzellularen Plasma. *Oester. bot. Zeitsch.*, vol. 54, p. 165.

MIGULA.

'95. Ueber den Zellinhalt von *Bacillus oxalticus* Zopf. *Arb. a. d. bak. Inst. d. tech. Hochsch. z. Karlsruhe*, vol. 1, p. 139.

MOORE.

:03. The Mitoses in the Spore Mother-cell of Pallavicinia. *Bot. Gaz.*, vol. 36, p. 384.

MOTTIER.

:03. The Behavior of the Chromosomes in the Spore Mother-cells of Higher Plants and the Homology of the Pollen and Embryo-sac Mother-cell. *Bot. Gaz.*, vol. 35, p. 250.

NADSON.

'95. Ueber den Bau des Cyanophyceen-protoplastes. *Scripta Botan. Horti Petropoli*, vol. 4.

NAKANISHI.

:01. Ueber den Bau der Bakterien. *Centralb. f. Bak.*, pt. 1, vol. 30, pp. 97, 145, 193, 225.

OLIVE.

:04. Mitotic Division of the Nuclei of the Cyanophyceæ. *Beih. z. Bot. Centralb.*, vol. 18, p. 9.

PALLA.

'93. Beitrag zur Kenntniss des Baues des Cyanophyceen-protoplasts. *Jahrb. f. wissensch. Bot.*, vol. 25, p. 511.

ROSENBERG.

:04. Ueber die Individualität der Chromosomen in Pflanzenreich. *Flora*, vol. 93, p. 251.

SCOTT.

'88. On the Nuclei in Oscillaria and Tolypothrix. *Jour. Linn. Soc., Bot.*, vol. 24, p. 188.

STEVENS.

'99. The Compound Oösphere of *Albugo bliti.* *Bot. Gaz.*, vol. 28, p. 149.

STEVENS.

:01. Gametogenesis and Fertilization in Albugo. *Bot. Gaz.*, vol. 32, p. 77.

TOWNSEND.

'97. Der Einfluss des Zellkerns auf die Bildung der Zellhaut. *Jahrb. f. wissensch. Bot.*, vol. 30, p. 484.

VAN HOOK.

:00. Notes on the Division of the Cell and Nucleus in Liverworts. *Bot. Gaz.*, vol. 30, p. 394.

VEJDOVSKY.

:04. Ueber den Kern der Bakterien und seine Teilung. *Centralb. f. Bak.*, pt. 2, vol. 11, p. 481.

WAGER.

'98. The Nucleus of the Yeast Plant. *Annals of Bot.*, vol. 12, p. 499.

WAGER.

:03. The Cell Structure of the Cyanophyceæ. *Proc. Roy. Soc. London*, vol. 72, p. 401.

WAGER.

:04. The Nucleus and Nuclear Division in the Root Apex of Phaseolus. *Annals of Bot.*, vol. 18, p. 29.

WOLFE.

:04. Cytological Studies on Nemalion. *Annals of Bot.*, vol. 18, p. 607.

ZACHARIAS.

:00. Ueber die Cyanophyceen. *Abhand. a. d. Gebiete d. Naturwissensch*, vol. 16.

ZACHARIAS.
:03. Ueber die Cyanophyceen. *Jahrb. d. hamburg. wissensch. Anstalten*, vol. 21, p. 49.

ZETTNOW.
'97. Ueber den Bau der grossen Spirillen. *Zeitsch. f. Hyg.*, vol. 24, p. 72.

ZETTNOW.
'99. Romanowski's Färbung bei Bakterien. *Zeitsch. f. Hyg.*, vol. 30, pl. 1.

ZUKAL.
'92. Ueber den Zellinhalt der Schizophyten. *Ber. d. deut. bot. Gesellsch.* vol. 10, p. 51.